SYNOPSIS IN MOLECULAR BIOLOGY

BY

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FOR

MEDICAL STUDENTS – POST GRADUATES



CONTENTS

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Molecular Biology

-What is meant by molecular biology ?

Introduction:

1- **Biochemistry:**
   Is science concerned with structural chemistry, metabolism, and biochemical informations of living matters?
2- **Genetics**:
   Is science concerned with understanding heredity and the expression of genetic information in molecular terms.
3- **Cell Biology:**
   Is science concerned with biological information of living cells and its nuclei:
   All above three terms coalesce together to yield the true **molecular biology**.

This chapter of molecular biology includes:

I) Structure and function of nucleotides (discussed before in part I).
II) Chemistry of nucleic acids (structure and biological functions).
III) Some genetic terminology.
IV) DNA synthesis (replication), mutations and DNA repair.
V) RNA synthesis (transcription).
VI) Control of gene expression.
VII) Cell cycle-Apoptosis-Carcinogenesis, and PCR technique.
VIII) Recombinant DNA technology, and DNA cloning.
IX) DNA mutations and repair.

Chemistry of nucleic acids

- Nucleic acids are polymer of nucleotides, there are two types of nucleic acids:

1- DNA: (mainly in nuclei) 2- RNA: mainly in cytoplasm.

- Nucleic acids are used for storage and transfer of information needed for protein production and expression of genetic informations.

- Nucleic acid of human cell [eukaryote cell] is present in nuclei with nuclear membrane; the nuclei is differentiated into 46 chromosomes, so DNA is involved in:
    - a- **Translation** of genetic information by synthesis of mRNA (transcription).
    - b- **Cell division** (replication).

- Nucleic acid of bacterial cells (prokaryote cell) is not enclosed into nuclear membrane ; its nucleic acid is in the form of single chromosome or circular plasmid in cytoplasm.

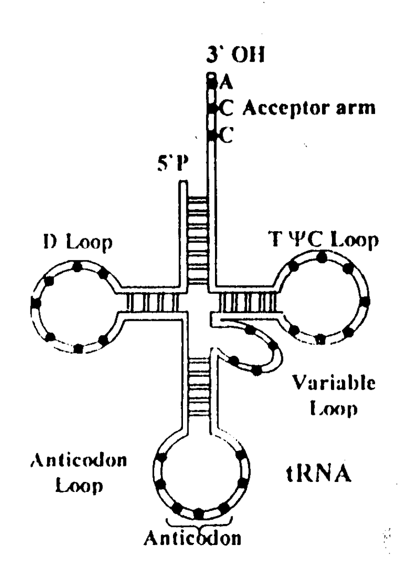
- **Site and functions of RNA nucleic acids:** (discussed before)

- RNAs are single stranded chains [mRNA 5%, tRNA 15%, and rRNA 80%] Synthesized in nuclei under control of DNA, then pass to cytoplasm where they participate in protein biosynthesis.

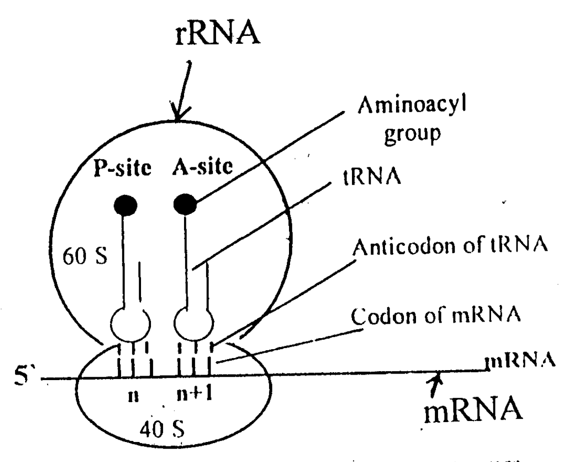
- Some viruses are single stranded RNA (ssRNA) as human immuno-deficiency virus (HIV), its genome is formed of two copies of ssRNA.

- The primer block of RNA is mononucleotide i [B.ribose, ii bases : guanine , cytosine, adenine, and uracil, and iii phosphoric acid].

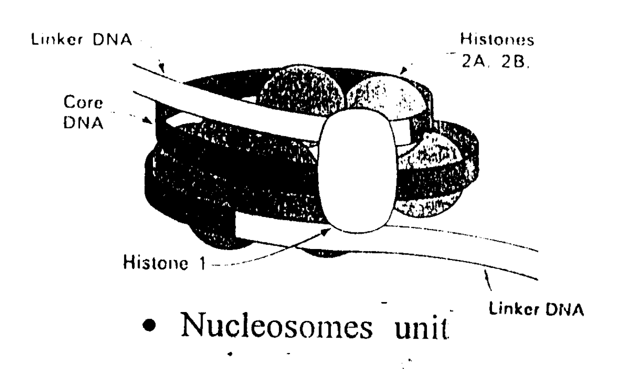
- DNA is nucleoprotein formed of long double stranded polynucleotides associated with basic protein [histone].



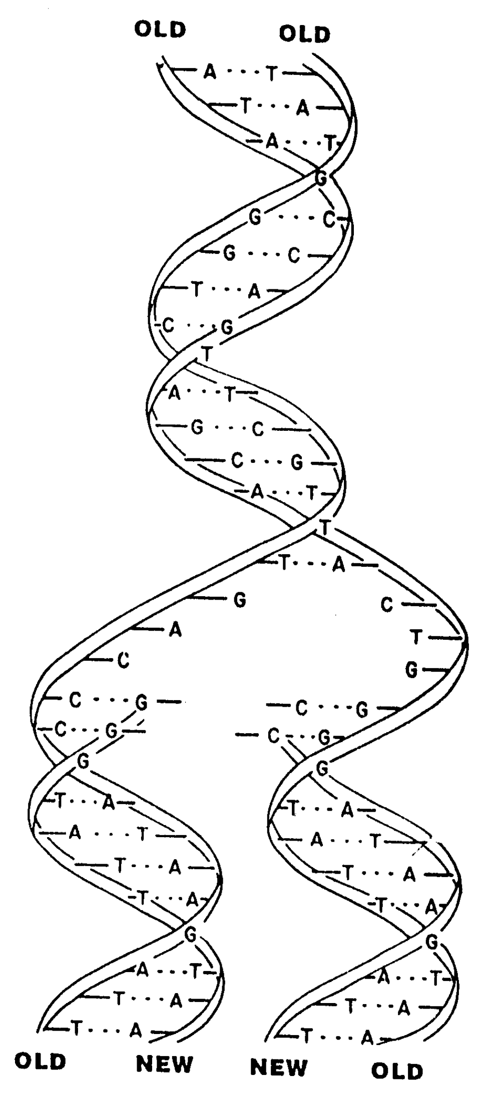
tRNA



This figure represents the interaction between the different types of RNA during protein synthesis. P-site and A-site are the peptidyl and amionacyl site respectively. Peptide bond is formed by the peptidyl transferase between the carboxylic group of amino acid in the P-site and the amino group of amino acid in the A-site.



- Nucleosomes unit



The double-stranded structure of DNA and the template function of each old strand (shaded) on which a new complementary strand is synthesized. (From James

DNA synthesis (replication)

- Its building block is mononucleotide [i B. deoxyribose , bases: guanine, cytosine, adenine, and thymine, in addition to iii phosphoric acid].

- The base is attached to carbon (1) of ribose.

- Phosphoric acid is attached to (-OH) group of C_5 of ribose, and to (-OH) of C_3 of another nucleotide ribose, so direction is 5' → 3' (OH) and named sense strand.

- The another strand is in direction of 3' → 5' (OH), and named antisense strand.
- So, the two strands run ant parallel .

- DNA is double stranded forms double helix, where guanine is attached to cytosine by 3 hydrogen bands, and adenine to thymine by 2 hydrogen bands, what is called the pairing rule.

- Mitochondrial DNA (mt DNA):

Is present in human as small double stranded DNA, mt.DNA is originated by ovum, so used as maternal lineage, its mutations are associated with certain types of myopathies.

- It forms less than 1% of total cellular DNA.

- DNA organization: (nucleosomes)

- The polynucleotides double stranded DNA is conjugated with eight octamer histone proteins.

- Histone proteins are basic proteins rich in lysine and argenine (positively charged) are united to the DNA (negatively charged) forming the DNA nucleoprotein (salt bond type).

DNA double helix

One nucleosome
(DNA+Histones)
=DNA nucleoproteins

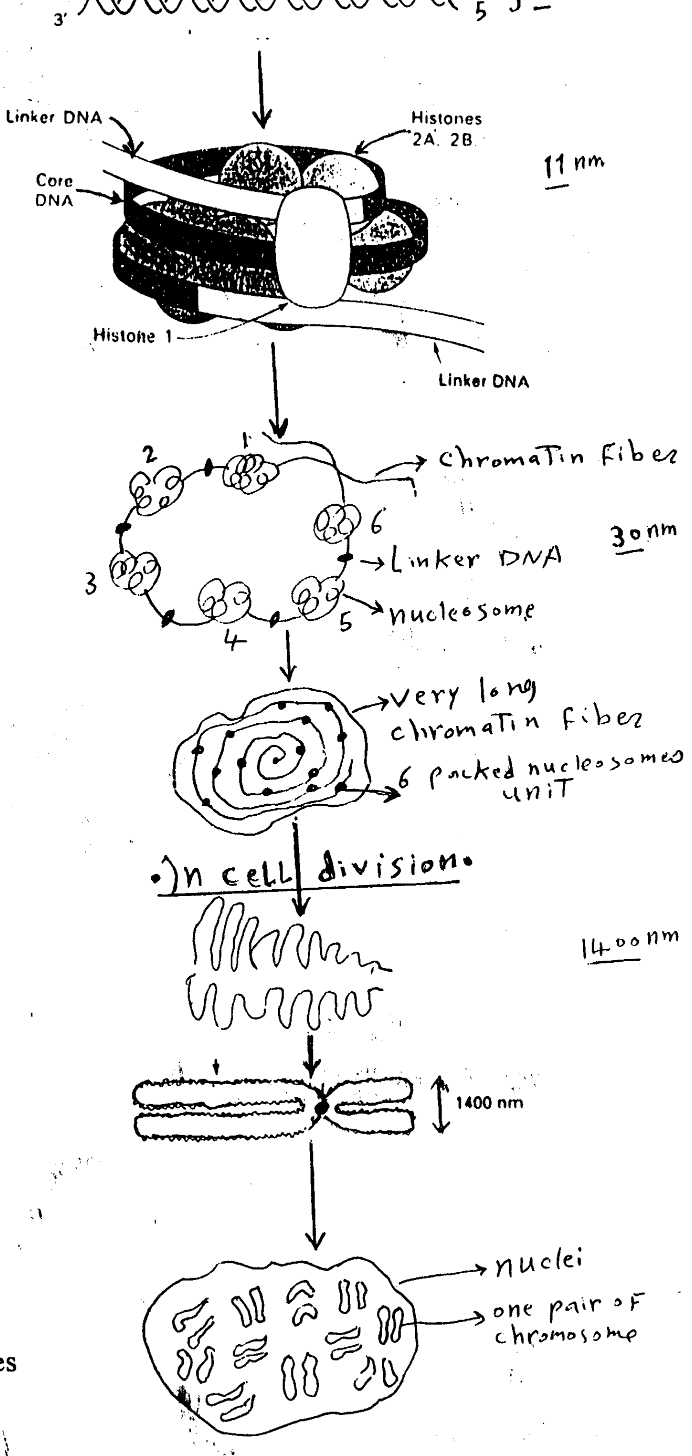
Repeated packed 6
Nucleosomes forming
Chromatin fiber

Human nucli with very long
DNA chromatin fiber
(very compact, condensed)
Its long about 2 meter
=whole cell genone

Condensed section of one
Chromosome

Metaphase of chromosome
Formed of two chromatids,
One from female and other
from male

Nucli in metaphase with
23 pairs (diploid) in somatic
Cells
And with 23 single Chromosomes
In gonadal cells (haploid) =
Whole genome.



- DNA organization

- The eight histone proteins (two copies of histone 2A, H.2B, H.3, and H.4 are (wrapped) surrounded by the DNA strand forming the nucleosome.

- Nucleosomes units are linked together by another one mole of Histone.

- The aim of this wrapping around histones is to condense and compact the very long DNA human strand (about 2 meters).

Chromatin fiber:

- More condense and compaction of nucleosomes occur by wrapping of 6 nucleosome units around a central channel of DNA strand.

- These units are repeated and packed forming the chromatin fiber (about 2 meters long in human nuclei).

- The whole chromatin fiber is organized into functional units called the genes (about 50-100.000 genes per cell).

- Chromosomes:

During cell division (metaphase), the chromatin fiber is arranged into 46 chromosomes, in human somatic cell [44 autosomes, and 2 sex chromosomes; XX in female and XY in male].

- Each human chromosome contains about 3500 genes in average.

- Chromatin DNA fiber must be compressed 8000 folds to generate a condensed chromosomes.

- Disorders of genes on chromosome X or Y lead to the sex or x linked diseases such as (hemophilia and favism).

- Disorders of somatic autosomal chromosomes lead to autosomal diseases as adult onset diabetes, juvenile onset diabetes (on chromosome 6) and sickle cell anemia (on chromosome 11).

- The genome: (whole DNA content)

- The somatic chromosome forms two identical chromatids (one from father, the other from mother).

- In gonads (ovary and testis) the whole chromosomes number is halved and called haploid genome (23 single chromosomes only).

- Whole DNA content of human somatic cell is called the diploid genome and contains about 7×10^9 base pair (bp), while in haploid (gonadal) genome is half (about 3.5×10^9 bp).

Genetic Terminology

- Gene:

- Is a part of DNA chromosome occupies a specific site [locus] on it.
- Its gene character is controlled by pair of genes (one from male, the other from female), each one is known as allele.
- DNA gene codes for single peptide chain (monocistrone).
- In human gene there is part called **exons**; which is coding part for protein synthesis.
- There is another non-coding part called **introns** → no protein synthesis.
- According to function, genes may be:
  i) Structural genes for protein synthesis [Hb or enzymes, ...].
  ii) Regulator (control genes) which modify the action of structural genes.

- The operon:

- Is system composed of structural genes for synthesis of specific catabolic enzymes, and is regulated by the control genes.

- Genome:

- Is total genetic content of a cell which is stored in DNA or cell chromosomes.

- The genetic code:

- Is relationship between the sequence (arrangement) of bases in DNA gene or codon of mRNA to the amino acids arrangement in peptide chain.

- **Chromosomes:**

- A thread like molecule of DNA (can be seen at cell division), situated in nucleus, and carry the genetic informations by much number of genes, the position of gene is called locus.

- Every one chromosome consists of two chromatids.

- Chromosomes are paired in 23 homologue (diploid cell) in somatic cells, but is single in gonadal cells [haploid].

- **Psuedogene:**
  - An inactive segment of DNA arising by mutation of a parental active gene.

- **DNA cloning:**
  - Is amplification of identical groups of cells or DNA in vivo.

- **Polymerase chain reaction: (PCR)**
  - Is amplification to millions of copies of specific DNA part in vitro using repeated cycles of denaturation and replication.

- **Proto-oncogene:**
  - Are genes present in normal cells, but if present in cancer cells or activated becomes malignant (can induce cancer).

- **Prokaryotes:**
  - Are simple cells present in bacteria or E.coli, have only one chromosome or circular plasmid in cytoplasm, it contains no nuclear membrane.
  - Its DNA codes for more than one polypeptide chain synthesis [polycistronic].

- **Eukarytoes:**
  - Are developed cells in mammals, fungi, and plants.
  - Its chromosome is located in nucleus.
  - Its DNA gene codes to one peptide chain only (monocistronic).
  - It contains nuclear membrane as in humans.

- **Plasmid:**
  - Is small circular cytoplasmic DNA molecule in prokaryotes [as E.coli].

  - It carries the gene information for antibiotic inactivation (cell resistance).

  - It can replicate alone away from DNA in another cell, so can be used in genetic engineering (DNA Recombinant).

- **Genotype:**

If the two gene alleles are identical, the genotype is homozygous for these genes.

  - If the two alleles are different the genotype is called heterozygous.

- **Phenotype:**
  - Is the physical or biochemical expression of the genotype (its appearance and characters).
  - Is affected by environment.

- **DNA unwinding:**
  - Is separation of the two double strands of DNA by specific enzymes (RNA polymerases).
  - It doesn't not occur in whole genome, but segment by segment.

- **DNA melting:**
  - Is separation of the two double strands by heating (melting temp.) where is the hydrogen bonds are broken.
  - If temp. is lowered again, there is binding of hydrogen bonds again [annealing without causing damage to DNA strands.

DNA Synthesis
DNA Replication

Is synthesis of new daughter DNA strands during cell division, and is complementary to the original (old) DNA strands.

- The reaction is catalyzed by DNA polymerases.
- During replication the double stranded DNA molecule, is separated into two strands.
- Each one is used as template for synthesis of a new daughter complementary strand.

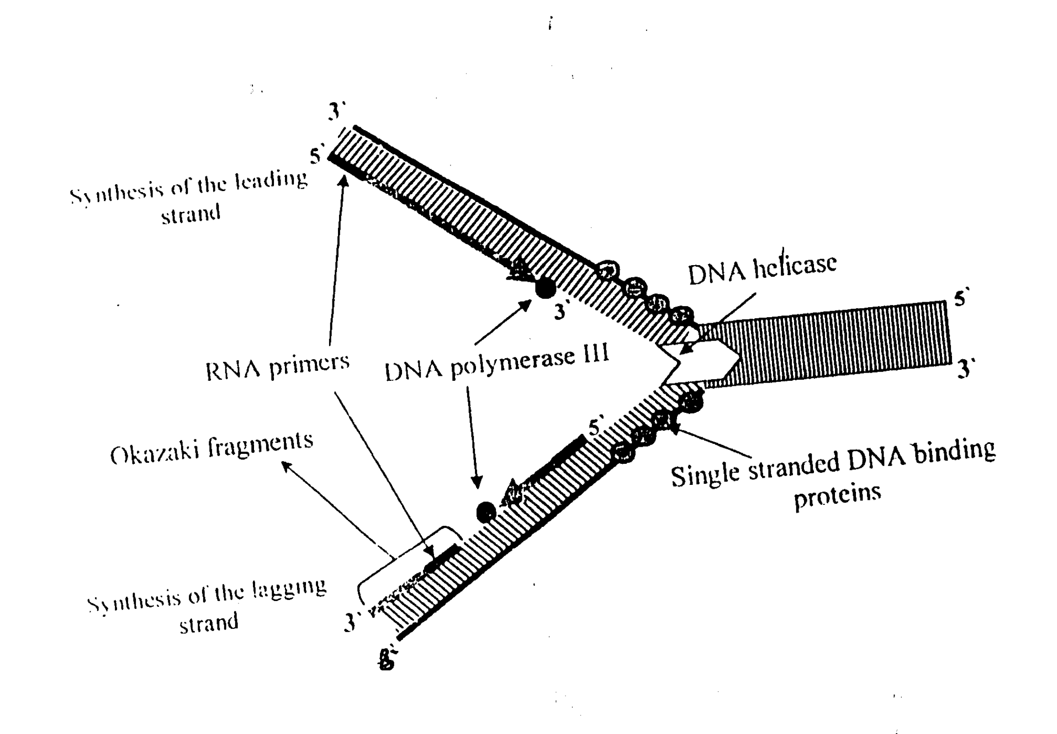
- Steps of DNA synthesis (replication):

I- strand separation (unwinding):

- The double stranded DNA molecule is separated from each other (separation not occur in whole molecule, but segment by segment).
- They form the V shape (replication fork).
- Separation process is carried out with aid of HD protein (Helix-destabilizing) which keeps the two strands separated.
- DNA helicase (rep protein is present near the centre of replication fork to forces its separation (it needs ATP).
- Topoisomerases relieve the super twisting [coiling of the non separated part of DNA strand].

II- synthesis of new DNA strands and its elongation:

- Each single original strand induces the formation of a second new identical strand, and is complement to it in order of guanine to cytosine and adenine to thymine, these reactions need:
    - a- DNA polymerases (I, II, III) in prokaryotes (bacteria) and DNA polymerases (alpha, beta, gamma) in eukaryotes (human).

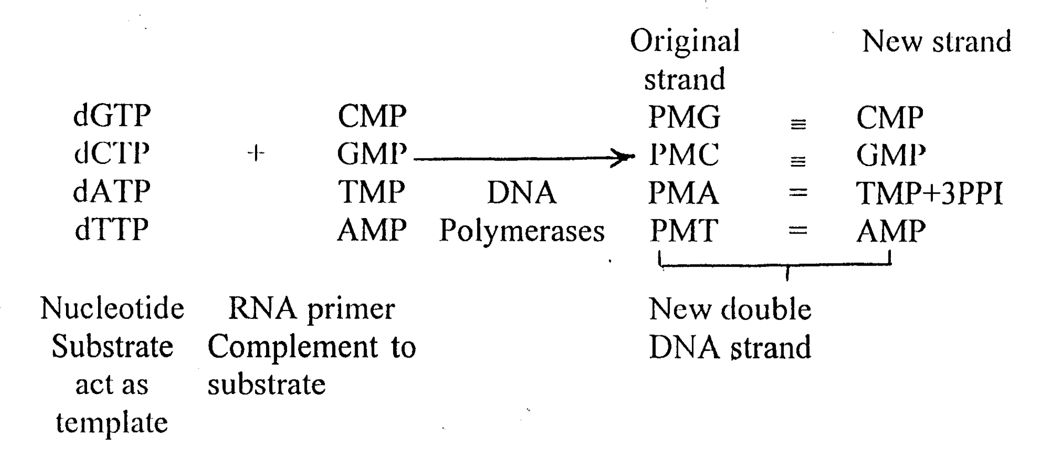


DNA synthesis (replication)

b- Four deoxynucleotides (dGTP, dCTP, dATP, dTTP) which are present free in nuclei and act as substrate and template for synthesis of new strand.

c- RNA primer: is short fragment of RNA, i.e., complementary to the DNA substrate.
RNA primer is synthesized by RNA polymerase under control of DNA.

- DNA polymerase III promotes and elongates daughter chain growth in direction of 5' → 3' toward the fork (leading strand).
- Synthesis of the other strand is in direction of away from the replication fork (lagging strand) and is disrupted by small fragments of DNA called **okazaki fragments** which are sealed (joined) by DNA ligase to form continuous strand.
- Fusion occurs between he original old single strand and the new synthesized DNA strand by aid with DNA polymerase I and DNA ligase.



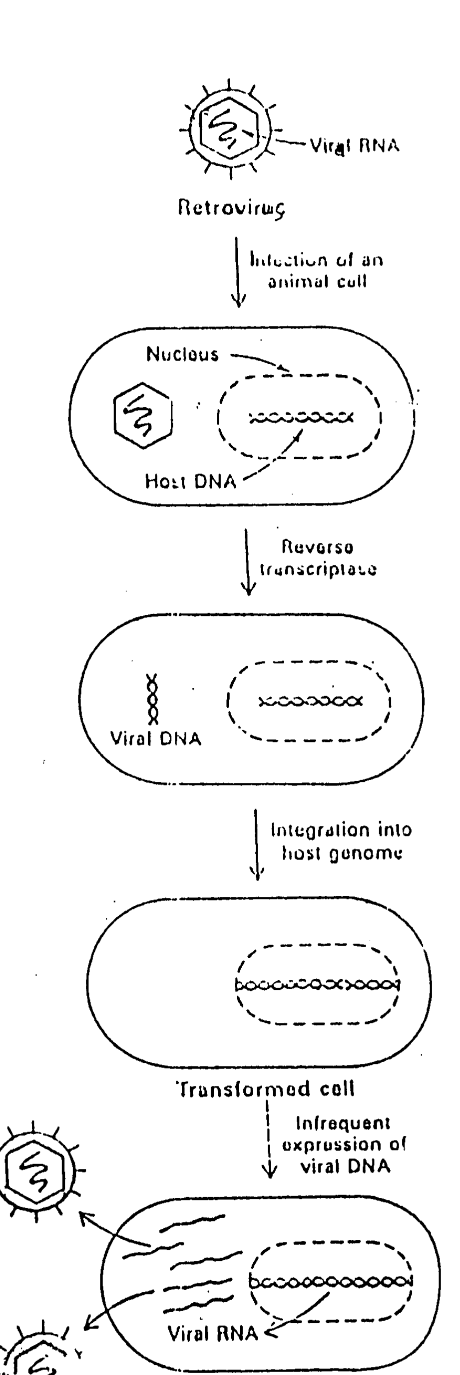
III) Excision (removal) of RNA primers and their replacement with DNA polymerase I, and stop action of polymerase III (stop replication process).
So, two identical daughter cells are arised from one parent cell.

Replication of RNA
[Reverse transcriptase]

- Some RNA viruses (AIDS, influenza, and hepatitis A) are called retroviruses, because they have reverse transcriptase enzyme (RNA dependant DNA polymerase) which has ability to synthesize DNA from single strand RNA (RNA → DNA), that is in reverse to normal (DNA → RNA → protein).

- Mechanism of replication:

- When retrovirus infects the cell, its single RNA strand acts as a template for synthesis of DNA strand complement to it, with aid of reverse transcriptase enzyme.
- dATP, dTTP, dGTP, and dCTP act as substrates.
- Then RNA strand in degraded (destroyed) by RNase enzyme.
- The formed DNA strand is replicated to two DNA strands by polymerase III, which can codes for viral RNA.
- The formed double stranded DNA enters the nucleus of infected cell and becomes included in cell genome which can replicate and produce many copies of viral RNA.
- It is considered a method of retroviruses multiplication in the infected cell.
- Reverse transcriptase are important in recombinant DNA technology.



ie of a retrovirus.

Replication of RNA
[Reverse transcriptase]

DNA repair

- DNA is damaged by a variety of physical or chemical agents leading to cell mutation → cancer, or cell death.
- Much of damaged DNA bases [on one strand] can be repaired and restored from the other strand, which is complement to it in order of [guanine → cytosine and adenine → thymine].

- Factors causing DNA damage:

I- Physical agents:

1- Ionizing radiation:

Such as gamma X Rays irradiation which produce high energy causing ionization of DNA bases.

2- unfiltered U.V sunlight (short U.V rays; near 260 nm) which forms the photoproduct thymine dimer.

*** Thymine dimer:**

Occurs in one strand of DNA (intrastrand) between two adjacent thymine bases in which carbon-6 of one thymine is attached to C4 of other thymine forming the thymine dimer covalent bond.

- Thymine dimer may cause cell mutagenesis, or cell death.
- Thymine-thymine dimer interferes with transcription of DNA replication beyond this dimer.

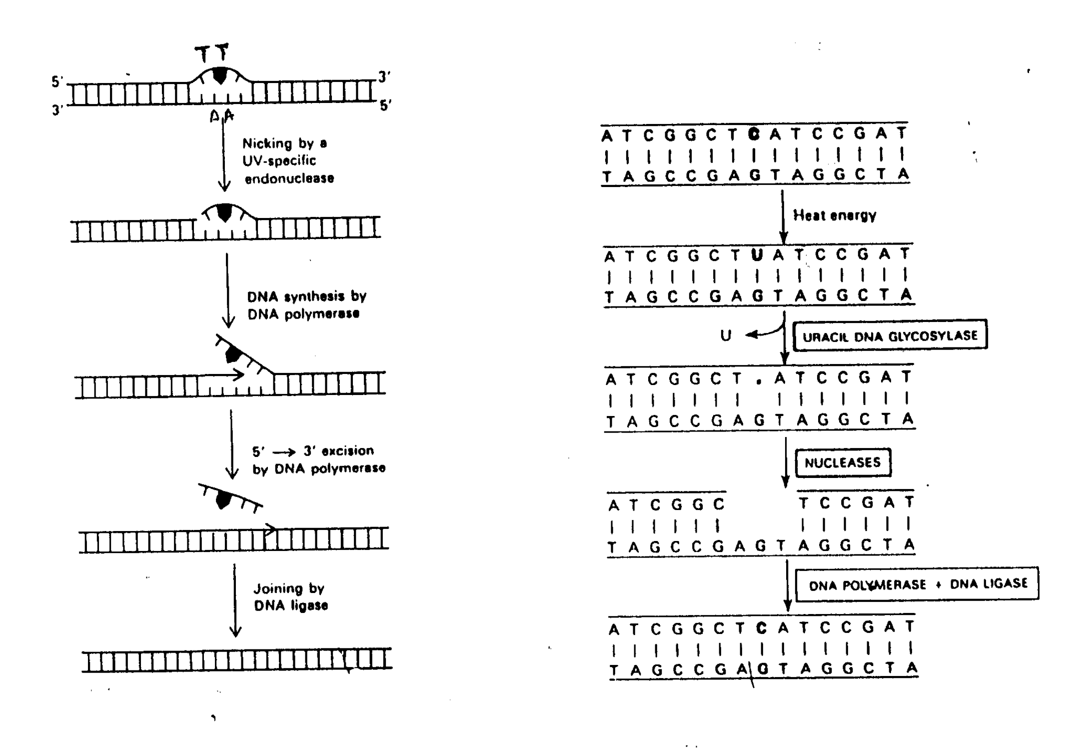
II- Chemical agents:

By alkylation's of bases in DNA (addition of methyl or ethyl group to the base → altered methylated base).

- Altered methylated bases → block DNA replication and proliferation → Cell death.
- Examples of alkylating agents:
  i) methyl nitrosourea ii) Ethyl methane sulfonate
  which are used as anticancer drugs (death of malignant cells).

Phosphate
pentose
Thymine 1
The link (dimer)
Thymine 2
Cyclobutyl ring

Thymine dimer



DNA repair of thymine dimer. (1)

Correction of base alteration (2)

- Mechanism of DNA repair of thymine dimer:

A- Direct repair: in prokaryotes by photo reactivation

The DNA photolyase binds to the dimer bond and ruptures it with aid of $FADH_2$ which translates the light energy electrons of visible blue light 370 nm (300-500 nm) to breaks the thymine bonds.

- The enzyme (Photolyase) is inhibited in dark after finishing its action.

B- Indirect repair (excision repair) in prokaryotes and eukaryotes:

By excision and replacing of damaged DNA thymine dimer with new normal segment using four enzymes.

Steps:

1- U.V specific endonueoleases which recognize the thymine dimer and makes nick(cut) near the dimer from its left 5' side.

2- DNA polymerase I for repair process by induction and synthesis of new DNA strand from the other intact strand which acts as template for the repaired one in direction of 5' → 3', and in order of adenine → thymine.

3- Exonuclease excise the thymine dimer region, and at the same time the newly synthesized DNA fragment is formed.

4- DNA ligase joins the newly synthesized fragment to DNA strand.

- **Xeroderma pigmentosa:**
  It is an autosomal recessive disease in skin due to absence of UV specific endonucleases.
- The skin suffers from keratosis and malignancy as melanoma in sun exposed areas during the first years of life.

C) Correction of base alteration:

- The bases on DNA can be altered spontaneously or by chemical compounds as nitrous acid which causes loss of amine group (--NH_2) from cytosine $\xrightarrow{-NH_2}$ uracil.

This alteration can be corrected as follows:

1- Specific glycosidase can recognize the altered bases and cleave them from the deoxyribose – phosphate backbone.
2- The specific endonclease DNA polymerase neck (cut) the DNA backbone adjacent to the altered base.
3- The DNA polymerase I insert new cytosine (complement to other intact chain).
4- DNA ligase repairs the sealed (cut) DNA strand.

**** important note:**

- If DNA repair is unsuccessful, the tumour suppressor gene (P53) triggers cell suicide (apoptosis) as protective mechanism to prevent proliferation of damaged DNA → prevents cell cancer.
- So, P53 gene acts normally as a molecular policeman in the cell.

Synthesis of RNA
[Transcription]

- General principles:

- Synthesis of RNA occurs in nucleus under control of DNA, with aid of RNA polymerase [DNA dependent RNA polymerases].
- One DNA strand acts as template for RNA synthesis and called the coding strand.
- The other DNA strand is called non coding strand.
- The RNA polymerase are attached to the DNA template, and does not leave it except after complete transcription.
- Transcription process resembles that of replication as follows:

  i) Initiation and unwinding (separation) of small part (segment) of double stranded DNA.

  ii) synthesis and elongation of RNA in direction of 5' → 3' in manner of guanine → cytosine, and adenine → uracil, with aid of RNA polymerases and nucleic substrates as (ATP, GTP, CTP, and UTP).

  iii) Termination using codon (UAA).

  iv) Post transcriptional modification to produce the mature RNA.

Eukaryotic RNA synthesis.

The basic mechanism of RNA synthesis is the same for all types of RNA (mRNA, rRNA, and tRNA) with aid of RNA polymerases.

- Types of polymerases:

1- RNA polymerase I:

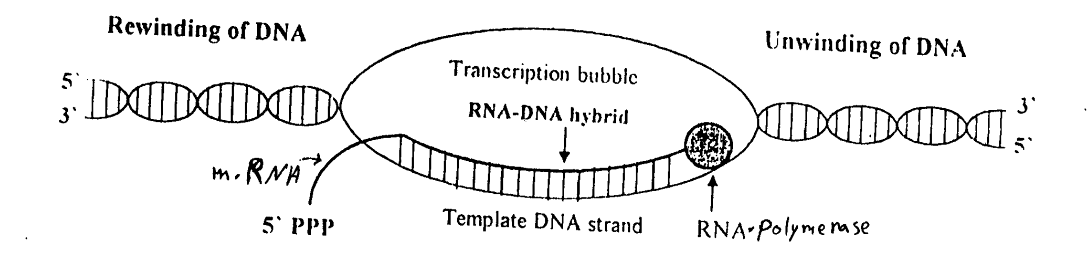
It transcripts rRNA.,

2- RNA polymerase II:

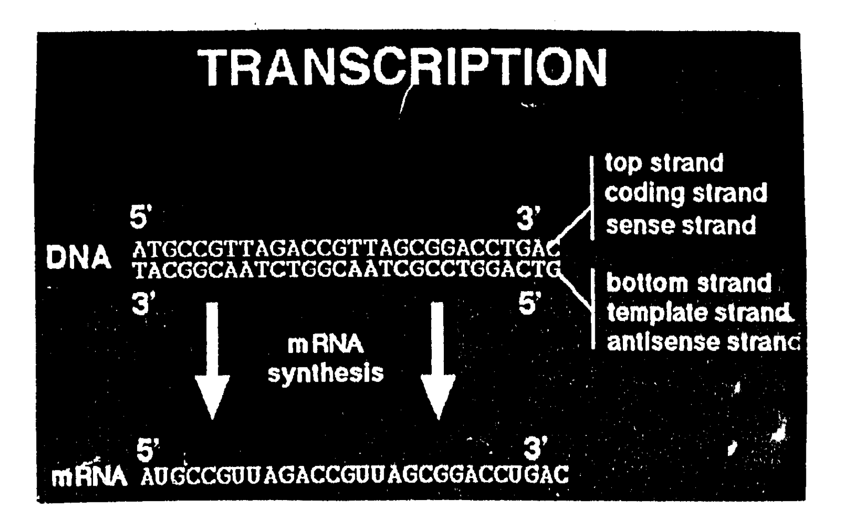
It transcripts mRNA.

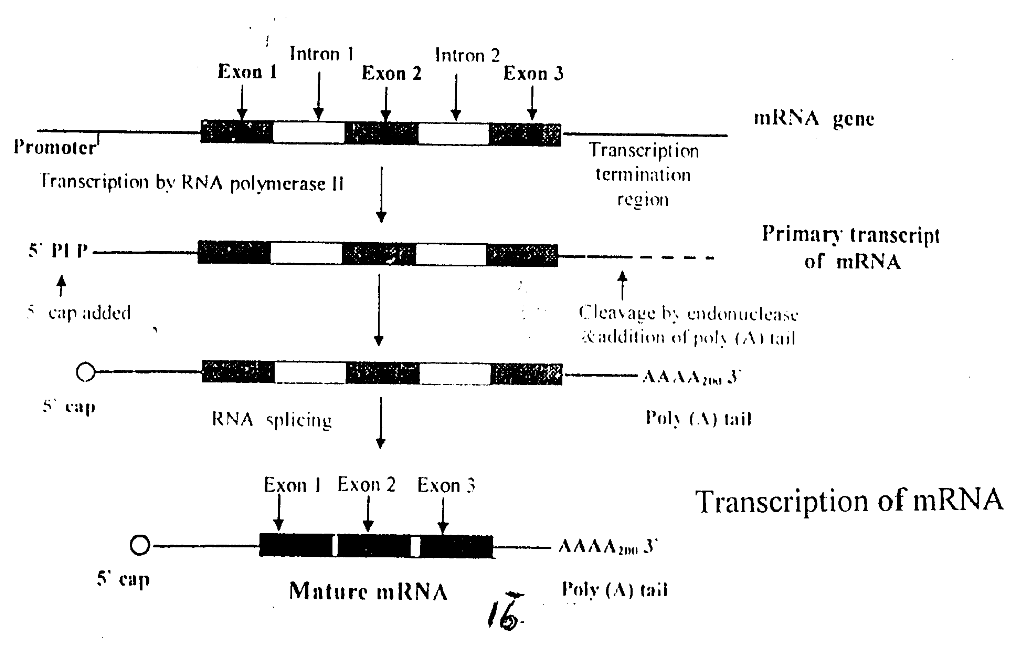
3- RNA polymerase III:

It transcripts tRNA



Synthesis of RNA
[Transcription]





Transcription of mRNA

Transcription of mRNA

It occurs in nuclei under control of DNA for coding of specific protein.

- Steps:

i) Initiation :

- The process begins with separation of small part of DNA (about 17 pb) with aid of RNA polymerase II. Which binds to specific region of DNA named as the promotion region.
- It starts with the initiate codon (AUG).

ii) synthesis and elongation:

- The coding DNA strand directs the synthesis of mRNA in manner of pairing role:
  Adenine → uracil and guanine → cytosine, and Adenine → Thymine, with aid of RNA polymerase II.
- The substrates of polymerases are nuclear GTP, CTP, ATP, and UTP.
- For elongation, RNA polymerase forms phosphate bond [connect 2 mononucleotides together] between –OH group of C_3 of one ribose, and –OH group of C_5 of the next ribose to forms dinculeotides.

iii) Termination:

Termination of synthesis is achieved by signal of the terminal codon (UAA).

- Post transcription modification of mRNA:

* transcribed mRNA in nucleus is called hnRNA, is modified in nucleus into mature mRNA by: Capping, addition of polyadenine, and splicing.

1) Capping:

- Methyl GTP is attached to 5' end (left side) of mRNA with aid of guanylyl transferase to protect this 5' end from attack by exonucleases.

2) Addition of polyadenine tail:

- mRNA requires about 40-200 adenine nucleotides added to the other 3' end of mRNA with aid of poly A polymerase enzyme, to protect the 3' end from exonuclease attack.

3) Splicing: (ligation)

- Primary (immature) mRNA is formed of many pieces, some of them will code for protein synthesis, are called **exons**.
- Others has no genetic information, not code for protein synthesis, are called introns.
- Splicing process includes removal of introns [non coding part] by endonucleases and ligation (splicing) of the exons together (protein coding part) by ligases → development of mature mRNA which then migrates to cell cytoplasm, for coding of protein synthesis (translation).

Protein Biosynthesis
[Translation]

Is formation of various body proteins, which are translated by specific sequence (arrangement) of nucleotides on mRNA, and is under control of DNA.

Examples:

- Liver & lymphocytes synthesize → plasma proteins.
- Mammary gland → milk proteins.
- Muscles → contractile proteins.

Requirements:

1- rRNA:

Is the cellular machine of proteins biosynthesis in cell cytoplasm.

- rRNA is formed of two subunits:
  - i) small rRNA unit (40S), in which mRNA is attached to it.
  - ii) Large rRNA unit (60S), in which tRNA is attached to it.
- Also there is two attachment sites:
  - (A) site: is acceptor of amino acids.
  - (B) Site for peptide elongation.

2- mRNA:

- mRNA has the genetic code for synthesis of the desired specific protein.
- Every three bases on mRNA form the codon which is complement to bases on tRNA (anticodon) in order of guanine → cytosine and adenine → uracil.
- The first 3 bases on mRNA (AUG) form the initiating codon which codes for the first amino acid (methionine), and acts as start signal for protein synthesis.
- The last codons is called non sense codons which doesn't code for protein synthesis as (UGA, UAG, or UAA).

3- tRNA:

- Are about 20 types, on for each amino acid.
- The activated amino acid is attached to ribose of its terminal adenine nucleotide at the 3 end (ACC).
- The 3 bases on central bulb side of tRNA form the anticodon, which is complement to mRNA codon.

4- Activated amino acids:

- Amino acids are activated (and attached to adenine of tRNA) by ATP, and amino-acyl-tRNA synthetase → activated amino acid – AMP complex.

5- Enzymes and other cell factors:

a) Enzymes:

i) Amino acyl-tRNA synthetase with ATP to activate amino acids and coupling to tRNA.

ii) peptidyl transferase with GTP for formation of peptide bonds, and chain elongation, then separation of formed peptide chain.

iii) Translocase with GTP to translocate peptide-tRNA complex from site (A) to site (B), thus leaving (A) site free for another tRNA in elongation stage.

b) cell factors:

i) Eukaryotic initiation factors (eIF):

eIF_3: binds mRNA to small rRNA

eIF_2: binds meth. tRNA and GTP to mRNA codon.

eIF_1: help binding of tRNA complex to codon on ribosome.

ii) Elongation factors:

EF_1: binds second tRNA and GTP to (A) site on ribosome.

EF_2: Translocate second tRNA from site (A) → site (B) in presence of GTP

iii) Releasing factor:

eRF: releases formed peptide chain from site (B) in presence of GTP and peptidyl transferase

- Steps of translation: (protein biosynthesis)

I) Initiation stage:

- It starts by binding of mRNA to the small ribosomal unit (40S) in presence of (IF_3).
- The first codon (AUG) on mRNA acts as signal to start the process with the amino acid methionine from the left side of peptide chain.
- GTP and IF_2 are attached to meth. tRNA → ternary complex.
- This complex recognizes its codon (AUG) on mRNA in presence of IF_1.
- Then the 60S large ribosomal unit is attached with the small 40S unit → 80S unit in presence of GTP.
- Meth. tRNA is now attached to big unit ribosome on site (p), then the process of elongation starts.

II) Elongation of peptide chain:

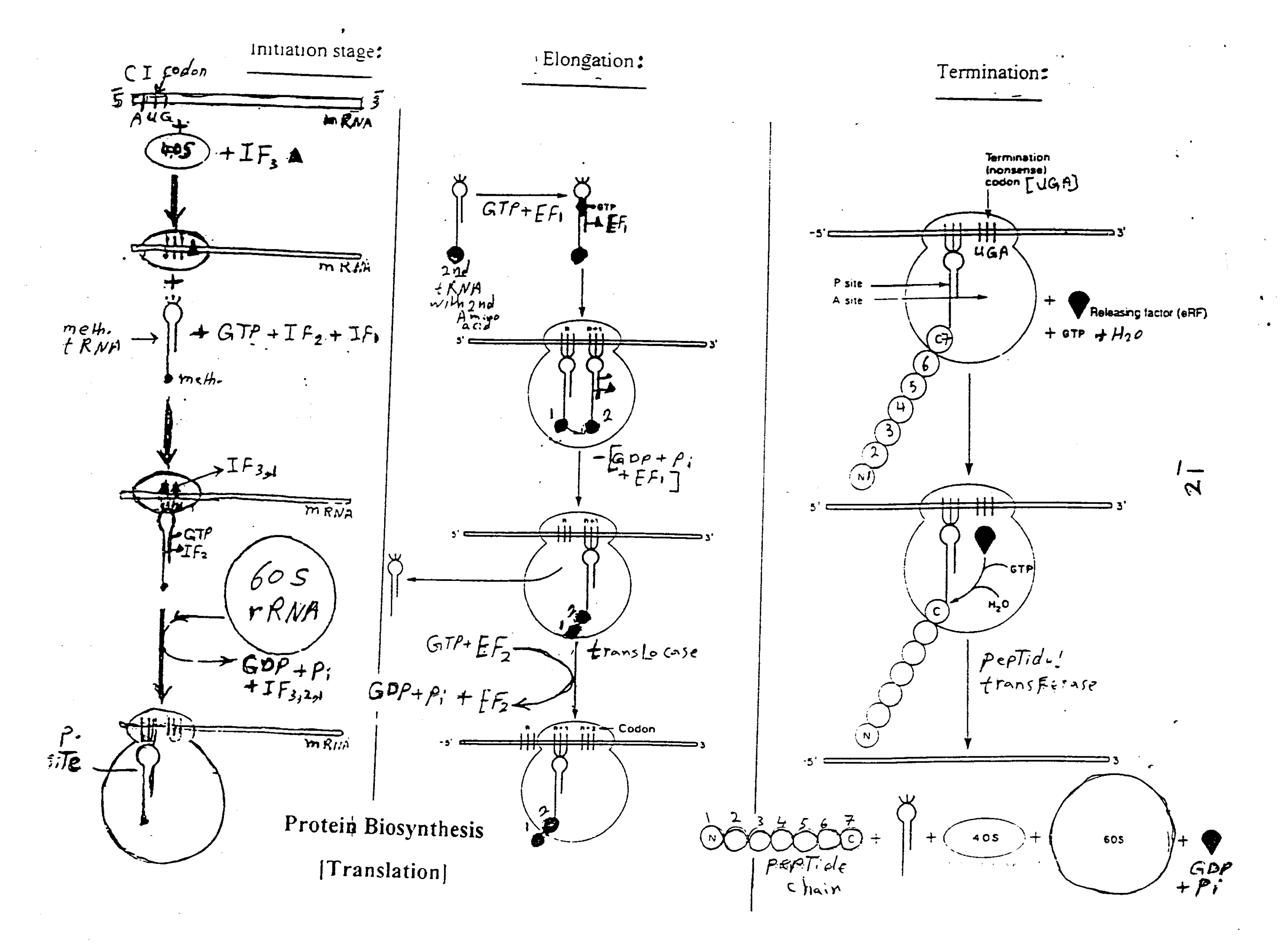
- The second amino acyl-tRNA is attached to GTP and EF_1 → Ternary complex, which enter the empty site (A).
- Then methionine on first tRNA is transferred to second amino acid on second tRNA by peptidyl transferase and GTP, and dipeptide is formed.
- After removal of methionine from first tRNA, it becomes uncharged [free], and ejected from the p site leaving it free, at the same time (GDP, Pi, and EFi) are released.

- Translocation:

- The peptide-tRNA in (A) site is translocated to the empty (p) site in presence of translocase enzyme, GTP, and EF_2.
- The (A) site now is free to third tRNA carrying the third amino acid, then GDP, Pi, and EF_2 are released.
- The process continues and repeated until complete peptide chain is formed.

III) Termination:

- After multiple cycles, the peptide chain is completed and released.
- The terminating non sense codons on mRNA (UAA, UAG, or UGA) appear in (A) site.



Protein Biosynthesis

[Translation]

- **Releasing of formed peptide chain in (p) site requires:**
  i) peptidyl transferase ii) GTP iii) RF to rupture the bond between peptide chain and tRNA in (p) site.
- Peptide chain is released in addition to GDP, Pi, tRNA, RF, and 80S ribosome which dissociates into 40S, and 60S subunits.
- A single mammalian ribosome can synthesize 100 peptide bonds each minute.

Post-translational modifications of protein biosynthesis

It occurs after translation (protein synthesis) to render them active.

1) Conversion of inactive protein to active one:

i) Some enzymes as pepsinogen is secreted inactive, and acted upon by hydrolytic proteases → remove small peptide part → active pepsin.

ii) Pre. Pro insulin (inactive) is changed into active insulin by removal of its (C) peptide chain → active insulin.

2) Glycosylation:

Occurs in Glogi apparatus, by conjugation of C_1 of carbohydrates to protein chain → active glycoprotein.

Types:

i) O. linked glycoprotein residue:
In which C_1 of carbohydrate is linked with (-OH) group of serine in peptide chain.

ii) N. linked glycoprotein residue:
In which C_1 of carbohydrate is linked to ($-NH_2$) group of basic amino acid aspargine in peptide chain.

3) Phosphorylation:

It occurs on (OH) groups of serine, theronine, or tyrosine of peptide chain: as

[glycogen phosphorylase (inactive protein enzyme) $\xrightarrow[ATP]{+P}$ active glycogen phosphorylase –P which stimulates glycogenolysis] .

4) Hydroxylation:

Is addition of (OH) group to proline $\xrightarrow[vit\ C]{in\ collagen}$ hydroxyl proline for collagen synthesis.

5) Carboxylation:

Carboxylation of gamma (γ) position of glutamate in prothrombin protein → gamma carboxyglutamate which allows binding of calcium ions → blood clot.

Regulation of Gene expression
(Regulation of protein biosynthesis)
The operon system in prokaryotes

The term operon system is a complete regulatory unit which includes number of nucleotides (genes) that code for synthesis of numbers of protein enzymes in prokaryotes.
It occupies a small part of DNA. Chain.

- Structure and function of operon system:-

The operon is formed mainly from:

1) structural genes (SG):

Consist of 3 genes (G_1, G_2, G_3) for synthesis of protein enzymes (such as glycolytic enzymes and lactose enzymes) through synthesis of mRNA.

2) Operator part:

Gives orders to structural genes to transcript specific mRNA for the desired protein.

3) Promotor:

Is part of DNA that can bind RNA polymerase and initiates coding of mRNA for the structural genes (G_1, G_2, G_3).

4) Regulator gene:

which regulates and controls the whole process of biosynthesis, by coding for formation of protein called (repressor protein) through mRNA.

* Repressor protein binds to the operator part and prevents the transcription of the structural genes.

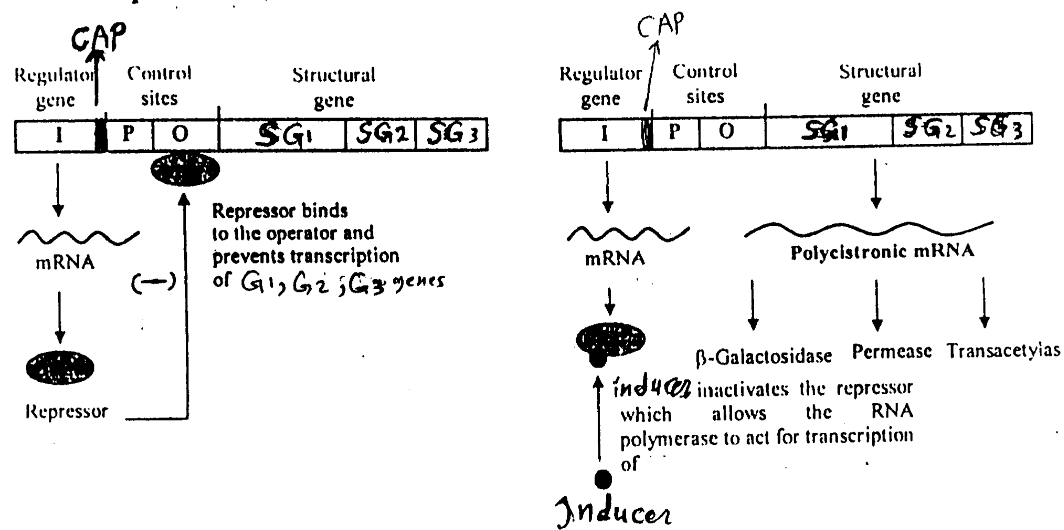
**** The inducer:**

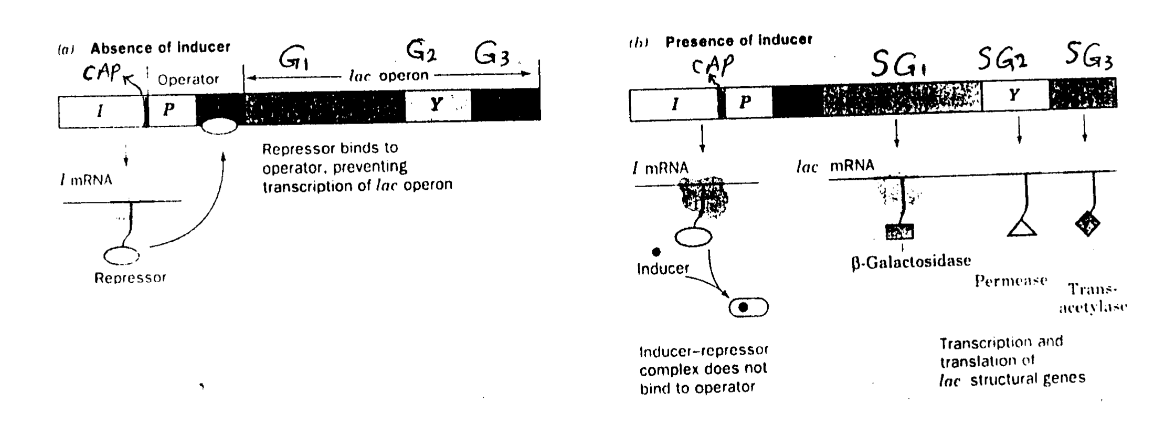
is substance that can bind with repressor protein forming repressor-inducer complex to prevent its attachment to operator, thus making it free to initiates the transcription of structural genes for protein biosynthesis (derepression).

Examples of inducers and repressors:

A) Glucocorticoids: act as inducers for gluconeogenesis enzymes in cases of hypoglycemia by stimulating synthesis of gluconeogenesis enzymes.

Lac Operon Model of E-Coli





Regulation of Gene expression

(Regulation of protein biosynthesis)

The operon system in prokaryotes

B) Insulin: is inducer of glycolytic enzymes in cases of hyperglycemia, but acts as repressor for gluconeogenesis enzymes at the same time to prevent hyperglycemia.

** CAP:

- Is catabolic gene activator protein, which binds to CAP site (between regulator and promoter parts) on DNA by aid of cAMP.
- Then CAP facilitates binding of RNA polymerase to promoter → increase transcription of operon system.

Mutations

- Is change in base sequence (arrangement) of a gene due to DNA mutation → alteration of genetic code (mRNA) → altered protein synthesis → inborn error diseases.
- The altered genes is called mutant gene.

- Causes:

1- Physical agents:

a) unfiltered U.V rays (short U.V rays; below 250 nm) may cause thymine dimmers.

b) X Ray and gamma rays may cause free radicles to be formed in tissues → formation of super oxide radicals → DNA damage.

2) Chemical agents:

eighty percent of mutations are environmental causes.

- Diet as Fungus Aflatoxin B toxin in peanuts.
- Occupational toxins as benzene, and asbestos.
- Polycyclic hydrocarbons as cigarette smoking.
- Some alkylating drugs as cyclophosphamide.
- Some oncogenic viruses: cause 15% of total human cancers.
    - i) DNA viruses: adenovirus, herpes virus, and hepatitis B virus.
    - ii) RNA viruses: retrovirus type C → leukemia and retrovirus B → mammary gland tumour.
- HIV retrovirus → AIDS.

Types:

I- point mutation (single base mutation):-

is mutation in one base only, which may be:

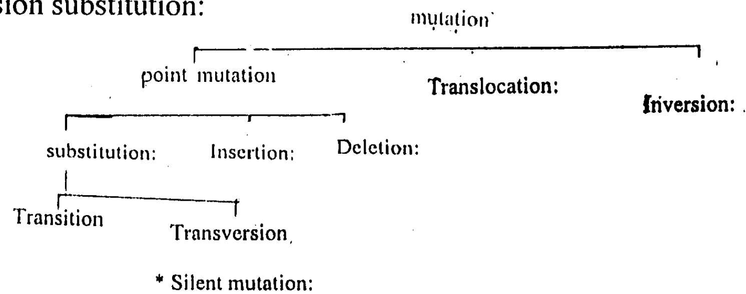
a) substitution:

Is substitution (exchange) of one base nucleotide by another, it may be:

i) Transition substitution:

Is change of one purine base by another purine base, or one pyrimidine to another pyrimidine base [CUT →UUT].

ii) Transversion substitution:



- Is change of one pyrimidine base (thymine) to another purine (adenine) on DNA gene related to B globin chain in Hb synthesis .
- This leads to change of normal codon for glutamate (GAA) on mRNA in 6^{th} position of B chain in Hb into → (GUA) which codes for valine → abnormal Hb S .
- Glutamate is polar amino acid, while valine in Hb S in non polar.

So, abnormal HbS is type of tranverse point mutation.

b) Insertion:

Is addition of one base or nucleotide in terminating non coding codes (as UAA) → synthesis of extra amino acid in peptide chain.

Non coding UAA $\xrightarrow[+Guanine]{mutation}$ GUA + A (adenine)
↓
Valine

c) Deletion:

Is loss of one already present base (or nucleotide) → change of the whole code frame sequence (arrangement) → major change in protein synthesis and function

CAA GGA GAG ←... original chain
CAG GAG AG ←.... other mutant chain

Insertion and deletion are serious, because whole code frame is changed (frame shift mutation), such as in thalassemia (complete absence of gene product).

II) Translocation:

Is movement of a piece (many nucleotides) of one chromosome to other non-homologous chromosome during DNA synthesis (replication).

III) Inversion:

Is inversion of large piece of DNA nucleotides within the chromosome.

*** Silent mutation: (not detectable mutation)**

Is type of point mutation, where the mutant gene is the third base which is not crucial for amino acid coding, because the most important is the first two bases.

[$\underset{UCU}{\text{|||}}$ is coding for serine $\xrightarrow[\text{in third base}]{\text{silent mutation}}$ $\underset{UCA}{\text{|||}}$ also codes for serine]

- Gene therapy of mutation: (in HbS)

- Is carried out by culture of normal bone marrow cells (containing intact normal B chain gene) with retrovirus which replicates the normal gene of B. chain.
- The new replicated normal B. chain gene is replaced in patient bone marrow → synthesis of normal B chain.
- This is the base of genetic engineering.

- Antenatal diagnosis of genetic disorders:

This carried out by aspiration of amniotic fluid in the 16^{th} week, then centrifuged:

i) The supernatant is used for estimation of α fetoprotein, if increased → spina bifida, or anencephaly.

ii) The amniotic cell precipitate are cultured for DNA studies for diagnosis of genetic disorders as Down's syndrome specially in female around 37 years.

- Preimplantation diagnosis of gene disorders:

1) Female ova, and male sperm are fertilized in vitro and cultured to 8 cells division phase.
2) DNA of fertilized ova is amplified by PCR to millions copies.
3) Analysis of that DNA in few hours to detect any gene disorders.
4) If Fertilized ova is normal, it may be implanted in mother uterus for normal pregnancy (artificial insemination).

Recombinant DNA technology
(Genetic engineering)

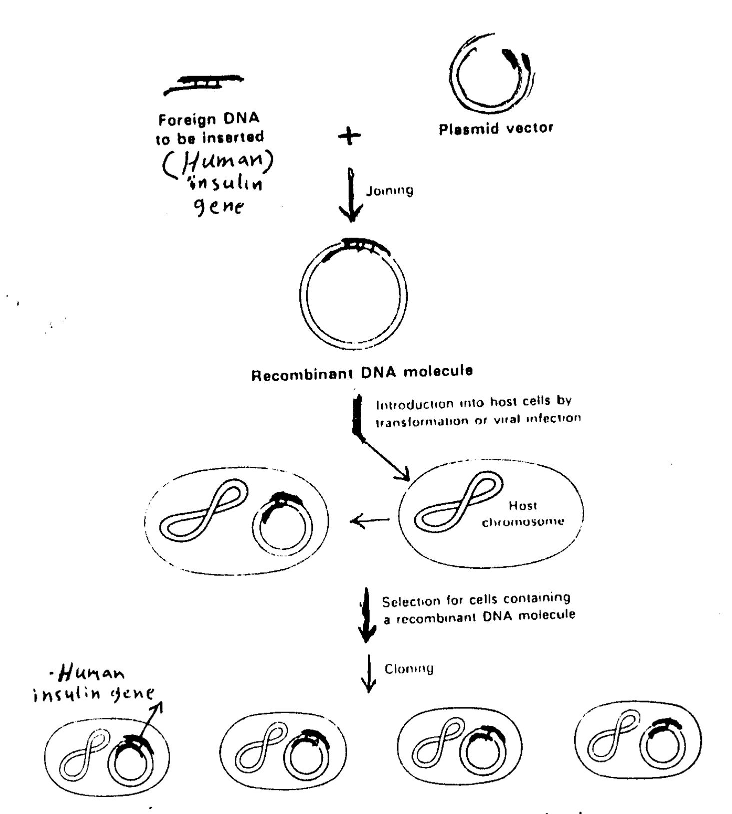
- Is the technique used for production of new DNA molecule composed of both human and bacterial DNA precursors.
- The new produced DNA molecule contains the genetic information of both human and bacterial DNA strands.

- Importance:

- Human proteins (as insulin, growth hormones) can be induced in abundance for human therapy.
- Recombinant DNA technology can be used for diagnosis and gene therapy in genetic diseases as sickle cell anemia, and thalassemia.
- The technique is used also in preparation of diagnostic Kits for AIDS and hepatitis B vaccine protein.
- Also in agriculture for good quality of plants and resistance for insects and bacteria.

- Concept of Recombinant DNA technology:

- Preparation of plasmid from bacteria (E.Coli).
- Plasmid is small circular duplex DNA, which can replicate away in other cells.
- It is used as vector carrier for foreign human DNA gene (Donor), and has the ability to replicate producing identical copies for human gene and plasmid itself (vector).
- The circular DNA plasmid (the vector) is cleaved at specific site by restriction endonucleases (enzymes produced by bacteria, which can cleave a specific short segment of DNA strand).
- A foreign gene (a piece of human DNA to be replicated, as insulin gene) is cleaved by the same restrictive endonucleases.
- The human foreign gene piece is inserted and recombined into the cleaved plasmid which is complement to it leading to formation of hybrid (mixed) plasmid, the process is catalyzed by DNA ligase.

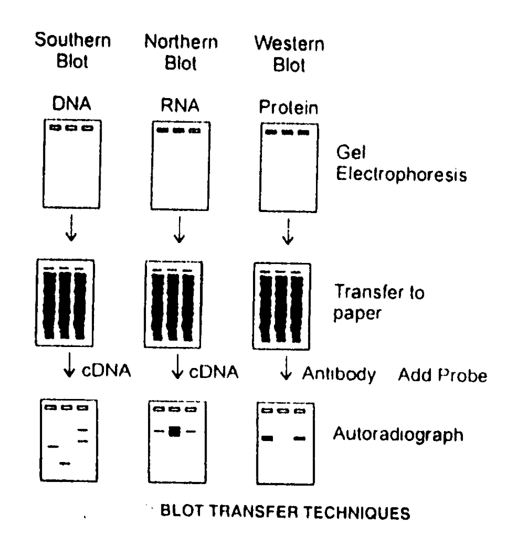


Synthesis and cloning of recombinant DNA molecules.

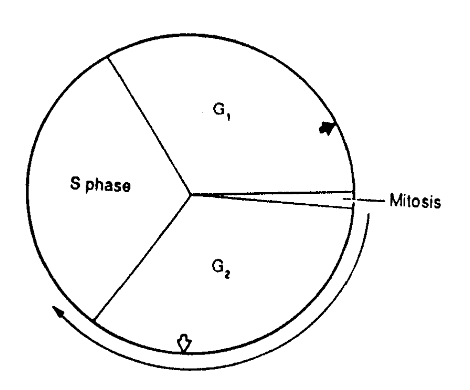
Recombinant DNA technology

(Genetic engineering)

- Then, the hybrid (recombinant) plasmid is introduced into host bacterial cells (prokaryotes), and by replication of host cells, a large numbers of copies (clones) of the plasmid and the human DNA piece (cloning), are produced .
- Replicated host cells are lysed and the new hybrid plasmids are isolated, and treated with the same restrictive endonucleases to release many clones [copies] of human insulin genes, which are isolated and purified for commercial uses.



Separation and Identification Of DNA



Mammalian cell cycle

Separation and Identification of DNA

- Analysis of DNA is called **southern blot,** in which radioactive probe (^{32}p) is used.
- Analysis of RNA is called **Northern blot,** in which radioactive probe is used.
- Analysis of proteins is called **Western blot,** in which specific antibody is used.

- Southern blot for DNA analysis:

- Is to characterize and visualize a specific DNA fragments among many mixed molecules.
- DNA samples (whole blood, sputum, tissues, ...) are treated and digested with restriction endonucleoses to yield different fragments of DNA samples.
- The DNA fragments are subjected to agarose gel electrophoresis, where DNA is migrated toward the cathode.
- Then transferred to a nitrocellulose paper in the same pattern in gel electrophoresis, and DNA fragments bind to cellulose paper by heat or U.V light → DNA denaturation → single stranded DNA.
- The arrangement of fixed DNA strands on cellulose paper are identified by the labeled radioactive probe which is complementary to DNA fragments on cellulose paper.
- Probe is already prepared known sequence of nucleotides, labeled with radioactive phosphorus (^{32}p) which can recognize a specific gene or act as cDNA [complementary DNA to mRNA].
- Then washing is carried out, and the DNA segments complementary to labeled probe are visualized by X Ray film.

Mammalian cell cycle

Cell cycle means cell division and formation of two daughter cells as a result of mitosis

Different phases of cell cycle:

1- Gap I phase (G_1):
   This phase lasts about (10 hours), is called resting phase (no mitosis), but there is protein synthesis.
2- Synthesis phase (S. phase):
   This phase lasts about 9 hours, there is DNA replication → 2 sister chromatid.
3- Gap 2 phase (G_2):
   It lasts about 4 hours, it is for preparation of mitosis.
4- Mitosis (m. phase):
   It lasts about one hour only, there cell division and two daughter cells are formed.
   Cell cycle is regulated by **cyclins** protein which binds to nuclear protein kinase which control the process of cell cycle.

Apoptosis

Apoptosis is a programmed cell death (cell suicide) according to a program which is beneficial to cell.

- Delaying of apoptosis may predispose to carcinogenesis.
- Apoptosis plays important role in aging, autoimmune diseases, and cancer development.
- It menstrual cycle, apoptosis is initiated in endometrial cells (death and shedding out) by fall levels of progesterone → menstruation.

Tumour suppressor genes

Are genes which encode proteins that inhibit cell cycle (mitosis), and stimulate apoptosis of oncogenic cells.

Therefore if there is proteins introduced into malignant cells, may cure cancer.

Examples of tumour suppressor genes:

1- P53 gene:

- P53 gene encodes for a protein P53 of molecular weight 53000, it is located on chromosome 17.
- P53 protein regulates genes of cell division.
- P53 protein binds to oncogenic virus proteins → Forms inactive complexes:
- P53 protein participates in cell death (apoptosis) if repair process of damaged DNA is failed.
- P53 protein acts as guardian of cell genome, acts as molecular policeman.

2- RBI gene: (Retinoblastoma gene):

- RBI gene is present on chromosome 13-
- It encodes for protein which suppresses tumour of retina (retinoblastoma).
- Inactivation of RB protein → small caner in lung prostate, retina, and connective tissues.
- RB proteins binds to certain viral proteins → inactivation.

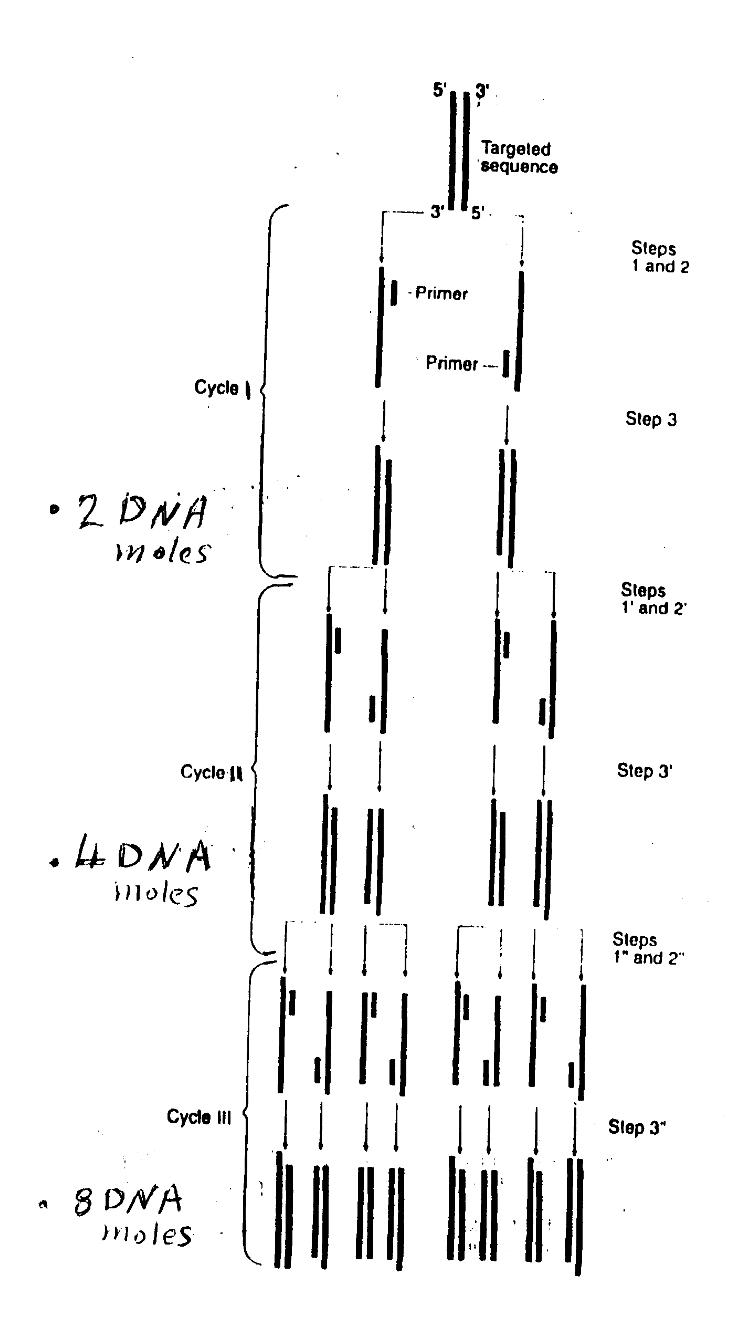
Polymerase chain reaction (PCR)

PCR is lab. Technique for rapid analysis of DNA by synthesis and amplification of minute amount of DNA to millions of copies for specific DNA gene.

Requirements:

1- The DNA samples:

DNA of cells, hair follicles, sperm viruses, bacteria, serum.



- Polymerase chain reaction: (PCR)

2- Two primers (already prepared) which are complementary to region of DNA strand at its end of the DNA sample.
3- Four deoxyribonucleotides (dATP, dTTP, dGTP, dCTP) whose act as substrates for DNA elongation.
4- Thermostable DNA polymerase for new DNA synthesis.

Steps:

1- Denaturation:

- The above requirements mixture is heated to 95°C for short time (30 sec.) to denature double stranded DNA sample → 2 single strands.

2- Primer annealing: (binding)

- The mixture is rapidly cooled to allow the two primers to bind at end of each DNA strand.

3- Elongation:

- The temperature of the mixture is raised to 72°C to allow DNA polymerase to elongate each primer along the single strand (complement to each other) → new double strand DNA.
- So, one original double strand DNA gives rise to two identical double strand DNA per one cycle.
- After 20 cycles, the original DNA is amplified a million fold, all of this takes less than one hour.

- Applications of PCR:

1- Detection of infectious viruses, even if present in minute amounts as in human immuno deficiency virus (HIV), hepatitis, and cytomegalovirus.
2- In forensic medicine to detect a tiny amounts of DNA from dried blood, hair follicle, or semen.
3- Diagnosis of genetic diseases, so we can detect any mutant gene.
4- In tissue typing in organs transplantation.
5- Production of humanized hormones (insulin) in large commercial scale by amplification of its gene by PCR → insertion into a suitable vector to be transcribed and translated to give humanized insulin hormone in commercial amounts.

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